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Contents of Volume 7

Numbers 3 and 4—Abstracts (Voir Autre Relivre)

Number 5, MARCH 1993

Number 6, APRIL 1993

Number 11, AUGUST 1993

Number 12, SEPTEMBER 1993

Number 13, OCTOBER 1993

Number 15, DECEMBER 1993

Thromboregulation: multicellular modulation of platelet reactivity in hemostasis and thrombosis

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ABSTRACT Blood platelets represent the first line of host defense when normal vessels are injured. Platelet adhesion to subendothelium, aggregation, and further platelet recruitment culminate in hemostatic plug formation, which is accompanied by the consolidating effect of fibrin deposition on and between platelets. The process is multicellular in that erythrocytes promote and neutrophils inhibit platelet plug formation. Endothelial cells in proximity possess three protective mechanisms (thromboregulators) for limiting the size of the hemostatic plug-ADPase, eicosanoids, endothelium-dependent relaxing factor/NO. We propose that in advanced atherosclerotic blood vessels such as coronary arteries, an ulcer or fissure in the fibrous cap of the atheroma serves as an agonist that transforms the platelet into a major prothrombotic offender. Induction of excessive platelet activation overcomes the normal thromboregulatory mechanisms. Erythrocytes further activate platelets, even in the presence of aspirin, and neutrophil blockage of platelet reactivity is insufficient to prevent impending vascular occlusion. Appreciating that multiple cell types and metabolic pathways are involved in modulation of platelet reactivity in vascular occlusion is a relatively recent concept. Strategies designed to restore processes such as thromboregulation may serve to improve therapeusis in thrombosis, which at present is far from optimal.—Marcus, A. J.; Safier, L. B. Thromboregulation: multicellular modulation of platelet reactivity in hemostasis and thrombosis. *FASEB J.* 7: 516-522; 1993.

Key Words: platelet activation and recruitment · transcellular metabolism · endothelial cell-platelet interactions · erythrocyte-platelet interactions · neutrophil-platelet interactions · endothelial ecto-ADPase · EDRF/NO

WHEN THE CONTINUITY OF A NORMAL blood vessel is interrupted, the initial response to the injury is defined as primary hemostasis (1). Superimposed on an intense vasoconstriction is the process of platelet activation. Platelets immediately adhere to collagen in the subendothelium that has been exposed by the injury. Collagen is a strong platelet agonist that induces platelet activation, leading to secretion of additional agonists. These include ADP, thromboxane (TXA_2),[2] and serotonin (5-HT). Secretion leads to recruitment of other platelets that aggregate upon the initial layer of adherent platelets, thereby forming the hemostatic plug (**Fig. 1**). Activated platelets also undergo a change in shape from a disk to a spiny sphere. In the process, rearrangement of membrane phospholipoprotein components forms a catalytic procoagulant surface. Activated coagulation factor X is bound to the platelet surface in the presence of activated factor V. Factor VII of the extrinsic coagulation system is also platelet surface-activated (1). These interactions culminate in formation of thrombin, which is the catalyst for fibrin transformation from fibrinogen, as well as being another strong platelet agonist. The thrombin-mediated reinduction of platelet activation and recruitment, along with coagulation, defines secondary hemostasis. The hemostatic platelet plug is now consolidated and impermeable as evidenced by complete cessation of hemorrhage (1). Thus, platelets form the nidus of a structure that withstands the back pressure induced by cardiac output. However, other cells are participating as promoters or inhibitors of platelet reactivity. The plug remains localized through this extracellular and fluid phase protein inhibitory activity as well as inhibitory mechanisms intrinsic to the platelet (2) (Fig. 1).

In contrast to normal hemostasis in injured but otherwise healthy vessels, arterial thrombosis in diseased vessels can be interpreted as a misdirected or abnormal consequence of the normal process. Although thrombi resemble hemostatic plugs morphologically (3), their development differs from that of hemostatic plugs in at least one important aspect. An arterial thrombus almost always occurs at sites of pathologic vascular damage. A major offender is the deeply fissured or ulcerated advanced atherosclerotic plaque in the vessel wall, which may contain an eroded fibrous cap with lipid-rich necrotic tissue and inflammatory cells (4–6). The agonistic potential of such lesions promotes far more platelet adhesion, activation, recruitment, and consolidation than an injured healthy vessel surface. In addition, lipid-rich arterial plaques are a nidus for intermittent thrombus formation due to recurrent fissuring as well as slow release of incorporated thrombin (5, 7, 8). The size of pathologic thrombi and the extent of associated normal tissue damage often surpass the capacity of normal control mechanisms. These pathologic thrombi can then extend or embolize to totally occlude vessels, leading to ischemia and death. The multiple factors involved in induction of arterial thrombosis and the associated myofibrotic response probably account for the difficulty in clinical management of these occlusive events (**Fig. 2**).

Several cell types in the microvasculature can modulate the growth of platelet thrombi. Microscopic time course studies of evolving thrombi initially demonstrate platelets adherent to subendothelium. The central core of the platelet

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[2]Abbreviations: TXA_2, thromboxane; 5-HT, serotonin; PGH_2, prostaglandin H_2; EDRF/NO, endothelium-dependent relaxing factor/nitric oxide; PGI_2, prostacyclin, prostaglandin I_2; 12-HETE, 12-hydroxyeicosatetraenoic acid; G protein, guanosine triphosphate-binding regulatory protein.

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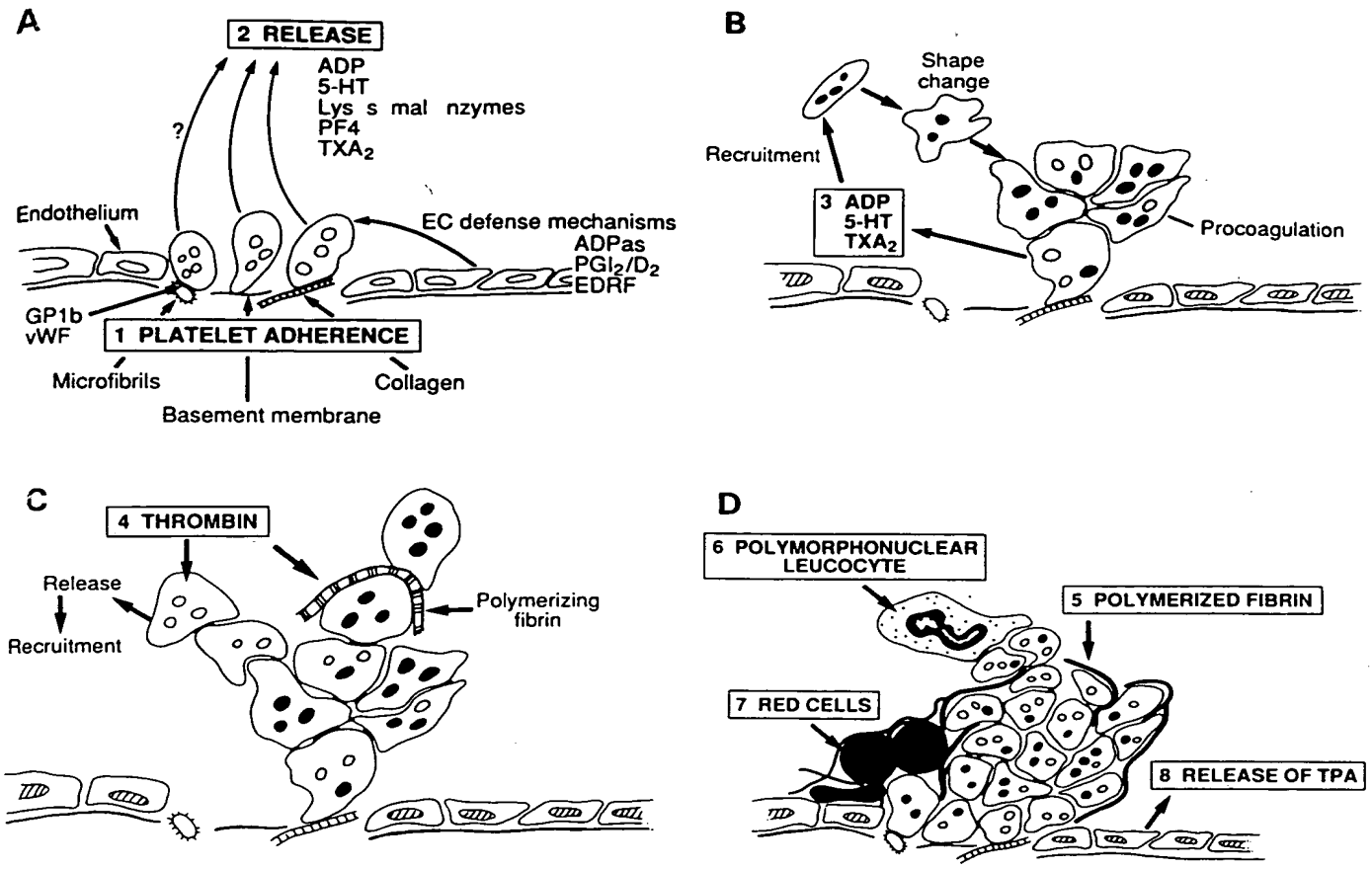

Figure 1. Diagramatic representation of the normal hemostatic process. *A)* Exposure of subendothelium due to vascular damage results in immediate platelet adherence to collagen, basement membrane and microfibrils in the presence of von Willebrand factor and platelet membrane glycoprotein Ib. The strong platelet agonist, collagen, induces release of ADP and serotonin (5-HT) from dense granules and several proteins from alpha granules. The eicosanoid thromboxane A_2 is also formed enzymatically from free arachidonic acid. Simultaneously, endothelial cell defense mechanisms (thromboregulators) become functional to limit the size of the thrombus. Concomitant with formation of the releasate, the recruitment phase is initiated. *B)* ADP, 5-HT, and TXA_2 are the most important recruiting components of the platelet releasate. They activate additional platelets arriving in the fluid phase, which then undergo shape change and aggregate onto the initial layer of activated platelets. Thromboxane and serotonin also induce vasoconstriction, which serves to limit the velocity of blood flow. Phospholipoproteins are now available on the platelet surface for catalytic activation of proteins of the coagulation system. *C)* Thrombin formation has several major consequences which lead to the final stages of platelet thrombus development. Platelet activation, release, and recruitment are reinduced and fibrin formation is initiated. Fibrin strands begin to intercalate between activated platelets and the thrombus becomes consolidated. *D)* The consolidated platelet thrombus is now virtually impermeable and its multicellular nature becomes obvious. There is in vitro and ex vivo evidence that intact metabolically viable erythrocytes are prothrombotic in that they increase the reactivity of activated platelets (12). Neutrophils are also seen in close contact with platelets. The modest chemotactic activity of released platelet 12-HETE may contribute to the arrival of the neutrophils. Platelet-neutrophil contact may be due to the adhesive platelet glycoprotein, P-selectin, interacting with its receptor on the neutrophil surface (33, 34). The neutrophil also down-regulates platelet reactivity and therefore may serve as an important antithrombotic modality (14). Formation of the platelet thrombus also signals the initiation of fibrinolysis, i.e., release of tissue plasminogen activator (TPA) from endothelial cells. (Adapted from Dr. J. F. Mustard with permission.)

thrombus gradually becomes admixed with erythrocytes, neutrophils, and subsequently, occasional monocytes. In addition to the aforementioned cells, there are normal metabolically viable endothelial cells adjacent to the lesion. For many years the presence of multiple cells in a thrombus was thought to represent a passive, random phenomenon. However, biochemical and functional interactions between platelets and other cells have been definitively demonstrated in vitro (9, 10) and there is strong evidence for their occurrence in vivo (11). Many of these interactions are thromboregulatory in that they culminate in enhancement or inhibition of platelet reactivity. For example, intact erythrocytes in close proximity to activated platelets markedly enhance platelet reactivity (12, 13). In sharp contrast, neutrophils (14) and endothelial cells (15–17) inhibit platelet activation and recruitment, thereby possibly limiting the size of the thrombus. As will be discussed, these interactions occur in the setting of cell proximity or direct contact. They can be due to secretory products of one or more cell types after activation by a specific agonist (or agonists) or to surface-connected modulators. In some circumstances, transcellular metabolism occurs in which the product of one cell is transformed by another. This can even result in the formation of a metabolite that cannot be synthesized by either cell alone. Occurrences of this phenomenon have thus far been demonstrated in vitro between platelets and endothelial cells, neu-

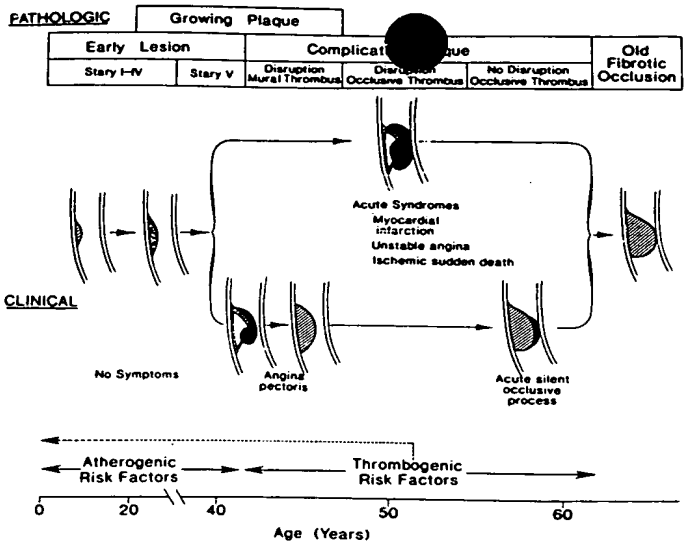

Figure 2. Progression of coronary atherogenesis to thrombogenesis on the pathologic and clinical level. Early lesions contain macrophages and lipid droplets. These progress to involve smooth muscle and a fibrotic lipid core. As the lesions progress to stage V they develop fissures and ulcerate. Intraluminal thrombi form and this constitutes the complicated plaque, eventually leading to the acute coronary syndromes. Repeated mural thrombi, initiated by activated platelets, result in progression of the atherosclerotic process. These concepts emphasize that atherogenesis and thrombogenesis should be studied in parallel. (Reviewed in ref 5.)

trophils, erythrocytes, and smooth muscle cells (9). These studies from our own and other laboratories suggest that the development and possible reversibility of thrombosis represent an integrated group of multicellular biochemical events (9).

BASIC BIOCHEMICAL PARAMETERS OF PLATELET REACTIVITY

Significant advances have been made in our comprehension of the biochemical events in platelet activation (2, 18). These mechanisms will be briefly summarized. Biochemically, the initial event in platelet activation is binding of an agonist such as thrombin to the extracellular domain of a specific platelet membrane receptor (19). As in other cells, platelet receptors for agonists and inhibitors are transmembrane proteins with cell surface and cytoplasmic components. The signal, initiated by receptor occupancy, is then transmitted by the cytoplasmic domain of the receptor, frequently through guanosine triphosphate-binding regulatory proteins (G proteins), to membrane-bound, signal-generating enzymes such as phospholipase C (**Fig. 3**). Activation of these enzymes induces generation of second messenger molecules. For example, phospholipase C initiates the phosphoinositide pathway of activation via hydrolysis of the membrane phospholipid phosphatidylinositol 4,5-bisphosphate (PIP_2). This gives rise to two platelet second messengers, inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DG). IP_3 induces calcium release from the platelet dense tubular system. A rise in free calcium in the cytosol is a critically important aspect of platelet activation and consequent events. Diacylglycerol activates protein kinase C, which in turn promotes protein phosphorylation, platelet granule secretion, and expression of the fibrinogen receptor on the platelet surface. Platelet cytoskeletal rearrangements that occur during platelet activation may be involved in granule secretion. As mentioned, the ADP released from platelet dense granules is important for recruitment of additional platelets to the thrombus. The fibrinogen receptor site is on the platelet membrane glycoprotein IIb-IIIa complex, which becomes transformed during platelet activation to assume its receptor function. The latter glycoproteins belong to the integrin group of adhesive protein receptors. By interacting with its receptors, fibrinogen forms a bridge between platelets in proximity, thereby resulting in platelet aggregation/cohesion (18).

A second important pathway in platelet activation is also initiated by enzymatic hydrolysis of a membrane phospholipid. Arachidonate, an essential fatty acid, is cleaved from phospholipid largely via catalysis by phospholipase A_2. Activation of this enzyme has been reported to involve several entities including the rise in free cytosolic calcium occurring during platelet activation, membrane Na^+/H^+ exchange, and one or more G proteins. Arachidonate cleavage can also result from combined actions of phospholipase C and diacylglycerol lipase (Fig. 3). Some arachidonate is released to the exterior of the platelet where it can be taken up by other cells and become involved in transcellular metabolism. In the cytosol, arachidonate is enzymatically oxygenated by cyclooxygenase and 12-lipoxygenase. The major biologically active platelet cyclooxygenase product formed by way of its transient intermediate, endoperoxide PGH_2, is thromboxane A_2 (TXA_2). This autacoid is released, acts as a vasoconstrictor, and takes part in the recruiting process via a platelet membrane receptor (Fig. 3).

CONTROL OF PLATELET REACTIVITY BY ENDOTHELIAL CELLS

Platelets in the circulation are not activated as long as the endothelium remains biochemically and physically intact. Actually, platelets produce a substance (or substances) that maintains the integrity of the vasculature and prevents spontaneous hemorrhage (1). When platelets do become activated as a consequence of injury, endothelial cells respond in a manner that is directed toward limitation or reversal of the consequences of platelet reactivity. This response can be defined as *endothelial thromboregulation.*

Thromboregulation is amenable to study in vitro. This is done with combined suspensions of cultured human umbilical cord endothelial cells and human platelets in motion in aggregometer cuvettes (15). Thus, maximal cell density is achieved and both functional and biochemical parameters can be measured simultaneously. Platelets become unresponsive to agonists in the presence of endothelial cell suspensions and this is due to at least three separate endothelial thromboregulatory systems. These include the eicosanoids, endothelium-dependent relaxing factor (now thought to be nitric oxide) (EDRF/NO), and the ecto-nucleotidase ATP-diphosphohydrolase (ATPDase), which has both ADP-ase and ATP-ase activities (17, 20–22).

Eicosanoids

The arachidonate pathway in endothelial cells can be initiated by agonists such as thrombin. The major product of eicosanoid metabolism in these cells is PGI_2, formed from the same endoperoxide PGH_2, which in platelets gives rise to TXA_2. Released endothelial cell PGI_2 can react with the platelet surface domain of a specific receptor, thereby initiating a G protein-linked signal transduction pathway of the

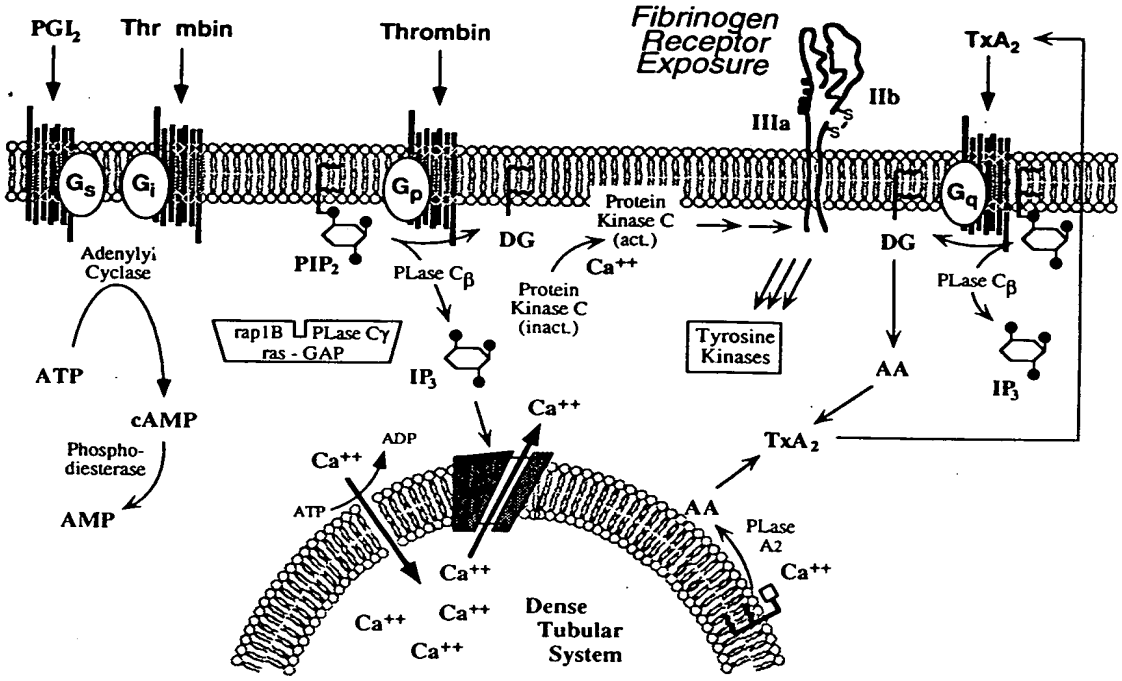

Figure 3. Signal transduction during platelet activation. The binding of agonists to membrane-spanning receptors on the platelet surface initiates cascades of intracellular second messengers, including inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DG). IP_3 releases Ca^{2+} from the platelet dense tubular system, raising the cytosolic free Ca^{2+} concentration. Diacylglycerol activates the serine/threonine kinase, protein kinase C, translocating it to the plasma membrane and triggering granule secretion and fibrinogen receptor exposure on the glycoprotein IIb-IIIa complex. At the same time, the rising cytosolic free Ca^{2+} concentration facilitates arachidonate (AA) release from phospholipids by phospholipase A_2, a process that may occur at both the plasma membrane and the dense tubular system membrane. Arachidonate is metabolized to thromboxane A_2 (TxA_2), which diffuses out of the cell, interacts with receptors on the platelet surface, and causes further platelet activation. At some point during this process, tyrosine kinases, including members of the src family, are activated in platelets and cause the phosphorylation on tyrosine of multiple platelet proteins, most of which have not been identified. Tyrosine kinase activation in platelets appears to occur predominantly as a consequence of fibrinogen receptor expression and platelet aggregation, but can also occur as an early step in platelet activation. In many cases, the interactions between agonists and the enzymes responsible for second messenger generation are mediated by a guanine nucleotide-binding regulatory protein (G protein). In platelets, G proteins have been shown to regulate phosphoinositide hydrolysis and cAMP formation, and are probably involved in the activation of phospholipase A_2. Phospholipase C (presumably phospholipase C_β) is activated in a pertussis toxin-sensitive manner by the still-unidentified G protein, G_p, and in a pertussis toxin-resistant manner by the G protein, G_q. Adenylylcyclase is stimulated by the G protein, G_s, and inhibited by the G protein, G_i. The G protein that regulates phospholipase A_2 activity remains to be characterized. Platelet receptors for thrombin, epinephrine, thromboxane A_2, and platelet activating factor have been cloned and shown to resemble other G protein-coupled receptors with a characteristic structure comprised of a single polypeptide with seven transmembrane domains. The low molecular weight GTP-binding protein, *rap*1B, has recently been shown to form a complex with phospholipase C_γ and *ras*-GAP, supplying a potential mechanism for regulating phospholipase C_γ activity. Other low molecular weight GTP-binding proteins may be involved in the regulation of vesicular transport and granule secretion in platelets. Provided by Dr. Lawrence F. Brass (18).

type described for activation of phospholipase C. In this case, the signal-generating enzyme is adenylyl cyclase. The latter catalyzes formation of the second messenger cAMP from ATP (Fig. 3). In contrast to other cells where it promotes reactivity, cAMP is a strong inhibitor of platelet function. cAMP antagonizes calcium-mediated responses by a variety of mechanisms (2). Effects of cAMP are generally thought to be mediated by a cAMP-dependent protein kinase (or kinases) (2).

Through a process of transcellular metabolism between platelets and endothelial cells, greater quantities of PGI_2 can be generated than can be synthesized by endothelial cells alone (15, 23). This is demonstrable by radiolabeling platelets with arachidonate and combining them with aspirin-treated, unlabeled endothelial cells that are incapable of producing endogenous endoperoxide PGH_2. Stimulation of these combined suspensions with thrombin, collagen, or ionophore results in production of radiolabeled prostacyclin plus other eicosanoids. The labeled PGH_2 intermediate required for formation of labeled PGI_2 can only originate from the labeled platelets. These results can be confirmed using unlabeled platelets and unlabeled endothelial cells, both of which have not been aspirin-treated. Prostacyclin is quantified by radioimmunoassay. In this system wherein endothelial cells produce PGI_2 endogenously as well as from platelet endoperoxides, more PGI_2 is synthesized than the endothelial cells can produce alone.

Endothelium-dependent relaxing factor/nitric oxide (EDRF/NO)

EDRF is an aspirin-insensitive fluid-phase autacoid produced by vascular endothelium and a large variety of other cells. It is identical with nitric oxide or a related nitrosyl compound. NO is the active principle of the group of compounds known as nitrovasodilators (24), and its mode of ac-

tion is via specific stimulation of the -containing soluble guanylyl cyclase in target cells. The resulting elevation of cyclic GMP blocks responsiveness of activated platelets. This inhibition is independent of that induced by cyclic AMP, but the two phenomena may be synergistic. In endothelial cells, NO is produced constitutively from L-arginine by the action of a calcium/calmodulin and NADPH-dependent NO synthase. In addition, NO has been identified in the central nervous system where it acts as a neurotransmitter, and in macrophages, where it is bacteriocidal (24–27).

Because EDRF/NO is a gas with a half-life of approximately 5 s, it is difficult to study in vitro. However, the system previously described for studying combined suspensions of endothelial cells and platelets in close contact is particularly suitable for this purpose. Both cell types can be aspirin-treated to inhibit PGI_2 formation, and inhibition of platelet aggregation can be readily demonstrated. Reversal of this inhibition by antagonists of EDRF formation and function can also be evaluated. Using this system, Broekman et al. (16) demonstrated EDRF/NO regulation of human platelet reactivity by cultured human endothelial cells. Platelet aggregation and serotonin release are inhibited in parallel in this aspirin-treated system. The endothelial cell-derived EDRF/NO activity is enhanced by superoxide dismutase, and reversed by hemoglobin and by competitive inhibitors of arginine metabolism such as derivatives of L-arginine with modified guanidino groups. When platelets and endothelial cells are separated by a permeable membrane and both cells stimulated by thrombin, the platelets remain unresponsive. Therefore the inhibition observed has been induced by a fluid-phase mediator in the absence of cell contact.

The significance of EDRF/NO as a major control system for vasomotor activity, platelet adhesion, and aggregation is becoming more apparent. Deficient EDRF/NO activity has been reported in atherosclerotic blood vessels, hypertension, vascular occlusion, and reperfusion injury (24).

Endothelial cell ecto-nucleotidase activity

The third endothelial thromboregulatory mechanism involves ecto-nucleotidases on the cell surface. Until recently, nucleotides were thought to serve mainly as intracellular energy sources. It is now appreciated that nucleotides can be released in response to cell activation or injury, and through specific receptors may elicit biological responses, some of which can be deleterious. An example of the latter would be the prothrombotic effects of excessive ADP release from platelets and injured tissues (28). The endothelial cell membrane thromboregulatory ecto-nucleotidases are aspirin-insensitive enzymes that metabolize released platelet ADP to AMP and adenosine, thereby limiting platelet recruitment (17).

ADPase activity is demonstrable in the in vitro aggregometry system described above for studying platelet-endothelial cell interactions. EDRF/NO effects and PGI_2 production by endothelial cells under study are blocked by simultaneous treatment with hemoglobin and aspirin, respectively. Nevertheless, in this setting inhibition of platelet responsiveness by endothelial cells persi his is observed by both aggregometry and measurement of platelet serotonin release. Such inhibition is largely due to ecto-ADPase activity on the endothelial cells. This can be established biochemically by incubating aspirin-treated endothelial cells with [^{14}C]ADP. Radio thin layer chromatography and aggregometry demonstrates a correlation between [^{14}C]ADP catabolism and loss of platelet stimulatory activity in the supernatant fluid. AMP accumulates transiently and is further metabolized to adenosine. The latter further inhibits platelet reactivity by elevating cAMP levels. An ADPase activity has been localized in the membrane fraction of human endothelial cells and is soluble in Triton X-100-ethylene glycol. Enzyme activity is detectable on polyacrylamide gels run in Triton, with a lead sulfide stain. The same protein band can hydrolyze ATP as well as ADP (21). Therefore the enzyme is an apyrase or ATP-diphosphohydrolase (ATPDase) (20).

Thus, at the present time there is in vitro evidence for at least three independent endothelial cell regulatory systems for platelet reactivity. As experimental horizons broaden and appropriate detection systems evolve, additional antithrombotic endothelial cell control systems may be identified. Only a few short years ago all the platelet inhibitory activity attributable to endothelial cells was thought to be due to prostacyclin alone. We now know that this is not the case. **Table 1** summarizes properties of the regulatory systems discussed.

ENHANCEMENT OF PLATELET REACTIVITY BY INTACT ERYTHROCYTES

Erythrocytes have long been known to play a role in hemostasis. Anemic patients have prolonged bleeding times that normalize upon correction of the erythrocyte deficit. It has been demonstrated that intact erythrocytes promote biochemical and functional responsiveness of activated platelets (12, 13). The platelet-erythrocyte interaction was carried out using a novel two-stage procedure. This permitted studies of cell-cell interactions and independent evaluation of platelet activation and recruitment within 1 min of stimulus addition. This assay system differs markedly from those usually used to study cell-cell interactions wherein cells are incubated together, the agonist is added, and results in the combined suspension monitored over a prolonged period of time. In the activation-recruitment assay, agonists are added to combined suspensions of platelets and erythrocytes and a cell-free releasate is prepared within 1 min. This releasate is then studied biochemically and is also added to a separate platelet preparation where it is tested for enhancing or inhibitory effects on aggregation. This in vitro action of the releasate serves as a prototype for the platelet recruitment process (12, 13).

Releasates obtained from mixtures of erythrocytes and platelets contain higher concentrations of secreted ADP (13) and induce greater aggregation (recruitment) than those ob-

TABLE 1. *Inhibitory thromboregulators associated with human endothelial cells*

Class	Type	Site of action	Aspirin sensitivity	Mode of action
Eicosanoids	PGI_2, PGD_2	Fluid phase autacoid	Inhibited	Elevation of platelet cAMP
Nitrovasodilators	EDRF/NO	Fluid phase autacoid	Insensitive	Elevation of platelet cGMP
Ecto-nucleotidases	ATPDase	Endothelial cell surface	Insensitive	Enzymatic removal of secreted ADP

tained from platelets alone. The erythrocytes remain capable of increasing platelet serotonin release (activation) despite aspirin treatment, enzymatic removal of released ADP, protease inhibition, or combinations of the above. An in vivo counterpart of these results is the possibility that erythrocyte enhancement of platelet reactivity can reduce the therapeutic effectiveness of aspirin. Erythrocytes also promote increased platelet arachidonate release and eicosanoid formation. Platelet stimulation with collagen or thrombin in the presence of erythrocytes results in significant increases in cyclooxygenase and lipoxygenase metabolites. In addition, activated platelet-erythrocyte mixtures with or without aspirin promote 3- to 10-fold increases in extracellular free arachidonate. The latter is available for transcellular metabolism in other cell types (12, 13). For example, released platelet arachidonate can be taken up by a stimulated neutrophil in proximity to form leukotriene B_4, the most proinflammatory eicosanoid yet described. In this way platelets play an indirect role in the inflammatory response.

The precise mechanism (or mechanisms) underlying erythrocyte enhancement of platelet reactivity has not as yet been delineated. The in vitro data on platelet activation and recruitment discussed here suggest that at least two separate events are occurring: Stimulated platelets activate a process in the intact erythrocyte, which causes the erythrocyte to further activate the platelet. The end result of this cell-cell communication is a prohemostatic and prothrombotic erythrocyte effect.

NEUTROPHILS DOWN-REGULATE HUMAN PLATELET REACTIVITY

Because the activation-recruitment system described above serves as an in vitro model for early cellular and biochemical events in thrombosis, it was used to study platelet-neutrophil interactions as well. During hemostasis, thrombosis, and the inflammatory response, the proximity of platelets and neutrophils allows for modulation of each others' activity. For example, we know that released platelet 12-HETE can be processed by unstimulated neutrophils to 12,20-DiHETE and 12-HETE-1,20-dioic acid, and by stimulated neutrophils to 5,12-DiHETE (29–32). On the other hand, the activation-recruitment system demonstrates that unstimulated neutrophils can modulate aggregation and secretion in stimulated platelets. When combined suspensions of normal numbers of neutrophils and platelets are stimulated by collagen or thrombin, platelet serotonin release and the proaggregatory activity of cell-free releasates from the mixture are markedly decreased (14). In these experiments, neutrophil serine proteases and EDRF/NO formation are not involved in the inhibitory activity. In the case of collagen, about 29% of the neutrophil inhibitory effect is attributable to ecto-ADPase activity, but this is not the major cause of the inhibition.

When cell-cell interactions are studied in the activation-recruitment system, the platelets and neutrophils are brought into close cell contact during the centrifugation step. Furthermore, upon platelet activation the adhesive protein P-selectin is expressed on the platelet surface, where it mediates adhesion to neutrophils (33, 34). It was important to discern whether P-selectin-mediated platelet-neutrophil adhesion was necessary for the inhibitory effect of neutrophils on platelets to occur. This question was examined with specific monoclonal antibodies to P-selectin, which blocked platelet-neutrophil adhesion. It is interesting that neutrophil inhibition of platelet reactivity was actually enhanced when adhesion was blocked. Neutrophils are more effective in controlling platelet reactivity when the cells are not metabolically adherent to each other. We propose that P-selectin acts in a prothrombotic manner because of its ability to reverse neutrophil inhibition of platelet reactivity.

Comparable results were obtained with the platelet 12-lipoxygenase product (12-HETE) or its derivatives originating from transcellular metabolism between platelets and unstimulated neutrophils. Like P-selectin, these lipoxygenase products reduced the inhibitory effect of neutrophils when the platelets were activated by thrombin (14).

In addition to participating in the inflammatory response accompanying the thrombotic process, neutrophils play a regulatory role. By inhibiting platelet reactivity, neutrophils may serve to limit the size and extent of the thrombus. Also, we do not know the precise functional implications of neutrophil metabolism of platelet lipoxygenase products. The process is efficient and well regulated and its significance may become more apparent when the properties of lipoxygenase-type eicosanoids are further elucidated (30, 31).

In summary, in vitro studies from many laboratories strongly suggest that thrombosis represents an integrated group of multicellular events. These events involve metabolic and functional interactions between platelets, endothelial cells, erythrocytes, and neutrophils. Future approaches to the pathogenesis and treatment of thrombosis will need to consider therapy that will affect more than one cell type and methods for enhancing activity of newly recognized thromboregulators. FJ

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